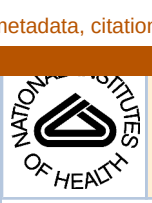

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Deletion of the 5-HT$_{3A}$-receptor subunit blunts the induction of cocaine sensitization

C. W. Hodge[*], **A. M. Bratt**, and **S. P. Kelley**

Departments of Psychiatry and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract

Serotonin (5-HT) receptors are classified into seven groups (5-HT$_{1–7}$), comprising at least 14 structurally and pharmacologically distinct receptor subtypes. Pharma-cological antagonism of ionotropic 5-HT$_3$ receptors has been shown to modulate both behavioral and neuro-chemical aspects of the induction of sensitization to cocaine. It is not known, however, if specific molecular subunits of the 5-HT$_3$ receptor influence the development of cocaine sensitization. To address this question, we studied the effects of acute and chronic intermittent cocaine administration in mice with a targeted deletion of the gene for the 5-HT$_{3A}$-receptor subunit (5-HT$_{3A}$ −/−). 5-HT$_{3A}$ (−/−) mice showed blunted induction of cocaine-induced locomotor sensitization as compared with wild-type littermate controls. 5-HT$_{3A}$ (−/−) mice did not differ from wild-type littermate controls on measures of basal motor activity or response to acute cocaine treatment. Enhanced locomotor response to saline injection following cocaine sensitization was observed equally in 5-HT$_{3A}$ (−/−) and wild-type mice suggesting similar conditioned effects associated with chronic cocaine treatment. These data show a role for the 5-HT$_{3A}$-receptor subunit in the induction of behavioral sensitization to cocaine and suggest that the 5-HT$_{3A}$ molecular subunit modulates neurobehavioral adaptations to cocaine, which may underlie aspects of addiction.

Keywords

5-HT$_3$; 5-HT$_{3A}$-receptor subunit; cocaine; conditioning; environment; locomotion; sensitization

Behavioral sensitization to psychostimulants refers to a progressive augmentation of responding associated following repeated treatment with amphetamine, cocaine or related drugs (Robinson *et al*. 1998). The process of sensitization is thought to produce enduring adaptive changes in brain and behavioral function that may underlie components of drug addiction (Kalivas *et al*. 1998; Robinson & Berridge 2000). Thus, behavioral sensitization is *in vivo* model in which progressive alterations in drug-induced behavior can be linked to changes in specific neurobiological systems (Carlezon & Nestler 2002).

In general, psychostimulant sensitization is mediated by a set of integrated brain structures termed the ‘motive circuit’ (Pierce & Kalivas 1997). This limbic circuitry, which includes the mesocorticolimbic pathways, is believed to gate and modulate appropriate behavioral responses to environmental stimuli. Although cocaine sensitization has been linked to a number of neurotransmitter systems within this pathway such as dopamine, γ-aminobutyric acid (GABA) and glutamate [recently reviewed by (Steketee 2005)], the precise mechanisms of drug sensitization remain to be fully characterized.

*Corresponding author: C. W. Hodge, PhD, Departments of Psychiatry and Pharmacology, Bowles Building, CB #7178, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. E-mail: chodge@med.unc.edu.

Evidence suggests that serotonin systems may play a role in behavioral sensitization to cocaine (Cunningham *et al.* 1992). Cocaine-induced motor activity was enhanced by pretreatment with the serotonin uptake inhibitor fluoxetine (Herges & Taylor 1998). Other studies have shown that 5-HT_3 receptor antagonists reduce cocaine-induced locomotion in the absence of effects on basal locomotion (Costall *et al*. 1990; King *et al*. 1997, 1998; Reith 1990; Svingos & Hitzemann 1992), and to attenuate hyperlocomotion induced by direct infusion of dopamine into the nucleus accumbens (NAcb) (Costall *et al*. 1987). Of particular interest to the present study, reports have indicated that 5-HT_3 antagonists block the development, or induction, of behavioral sensitization to cocaine (King *et al*. 1997) but have no effect on expression of sensitization tested after a period of withdrawal (Szumlinski *et al*. 2003). Accordingly, 5-HT_3 immunoreactivity is down-regulated in the shell of the NAcb during the induction of cocaine sensitization but not 2 weeks after withdrawal of cocaine (Ricci *et al.* 2004). These data suggest that 5-HT_3 receptors may mediate the induction but not expression of cocaine sensitization.

The 5-HT_3 receptor is a ligand-gated ion channel comprising a pentameric cylindrical-shaped structure surrounding a centrally gated ion channel (Barnes & Sharp 1999; Fletcher & Barnes 1998; Yan *et al.* 1999). 5-HT_3 receptors are known to be expressed in limbic brain regions, with dense binding found in frontal cortical layers II–III, amygdala, hippocampus and moderately dense binding found in the NAcb and striatum (Kilpatrick *et al.* 1996; Morales *et al.* 1998). To date, two channel subunits are known, the 5-HT_{3A} subunit (Belelli *et al.* 1995; Maricq *et al.* 1991), shown to be present in the hippocampus, amygdala, cortex and NAcb (Tecott *et al.* 1993); and the 5-HT_{3B} subunit (Davies *et al.* 1999), known to be co-expressed with the 5-HT_{3A} subunit in the amygdala, caudate and hippocampus in humans. Heteromeric assemblies of both subunits display physiological properties closely resembling those of neuronal 5-HT_3 channels (Davies *et al*. 1999). In rats, however, 5-HT_{3B} subunit transcripts are restricted to peripheral neurons (Morales & Wang 2002), which suggest that rodent neural 5-HT_3 receptors might be 5-HT_{3A} homomeric receptors or heteromeric receptors containing 5-HT_{3A} subunits combined with subunits other than the 5-HT_{3B} subunit. Indeed, comparison of electrophysiological properties of rodent neural 5-HT_3 receptors has shown the presence of receptors with both high and low conductances (Hussy *et al.* 1994; Jones & Surprenant 1994; Yang *et al.* 1992), which suggests the presence of at least two different 5-HT_3 receptor channels (Fletcher & Barnes 1998).

The role that specific molecular subunits of 5-HT_3 receptors play in the temporal development of cocaine sensitization has not been studied. The following experiments were designed to evaluate the effects of total absence of the 5-HT_{3A}-receptor subunit on induction of locomotor sensitization to cocaine.

Materials and methods

Mice

5-HT_{3A} receptor (−/−) mice were derived using homologous recombination as described (Zeitz *et al.* 2002). F_1 hybrid C57BL/6J × 129 heterozygous progeny were backcrossed with C57BL/6J mice to produce F9 generation congenics. Heterozygotes from the F9 generation were bred to generate wild-type and 5-HT_{3A} (−/−) mutants used in the present study. Only male mice were used in the present study. Genotyping was conducted in Dr David Julius' laboratory at the University of California, San Francisco as previously described (Hodge *et al.* 2004; Kelley *et al.* 2003; Zeitz *et al.* 2002). The experimenter conducting behavioral studies was blind to genotype. 5-HT_{3A} (−/−) and wild-type C57BL/6 littermate control male mice (age, 15–20 weeks; weight, 28–35 g) were used as test subjects for all behavioral studies. All animals were drug naïve prior to testing, and separate batches of mice were used for each experimental set. The mice were housed in plastic cages lined with Cell Sorb bedding and provided with food

(Harlan, Indianapolis, IN, USA) and water *ad libitum*. The vivarium was maintained on a 12 h light/dark cycle (lights on at 06:00) at a temperature of 22°C. All procedures were carried out in accordance with the NIH Guide to Care and Use of Laboratory Animals and institutional guidelines.

Locomotor apparatus

Locomotor activity was measured in Plexiglass chambers (43 cm^2) located in sound-attenuating cubicles equipped with exhaust fans that masked external noise (Med Associates, Lafayette, IN, USA). Two sets of 16 pulse-modulated infrared photo beams were placed on opposite walls at 1-inch centers to record X–Y ambulatory movements. Activity chambers were computer-interfaced (Med Associates) for data sampling at 100-millisecond resolution. Horizontal distance traveled (cm) was recorded for all sessions.

Locomotor ability and effects of acute cocaine

5-HT_{3A} (−/−) mice (n = 13) and wild-type controls (N = 10) were handled and weighed daily for 1 week prior to activity testing. Mice were transported from the adjacent vivarium to the test room and allowed to acclimatize for a period of 30 min prior to all testing sessions. Spontaneous locomotor activity and habituation to a novel environment was tested during 1-h sessions on three consecutive days. Subsequently, the effects of acute cocaine [0, 10.0 and 20.0 mg/kg intraperitoneal (i.p.)] were tested in all mice once per week. Cocaine doses were administered immediately prior to 15-min locomotor test sessions according to a Latin square randomized dosing design. Sessions were set at 15 min to limit exposure to the test chambers because other work has shown that 5-HT_3 receptors regulate contextual conditioning (Harrell & Allan 2003), which may influence sensitization (Robinson *et al.* 1998).

Locomotor effects of chronic intermittent cocaine

The effects of chronic intermittent cocaine were studied in wild-type (n = 7) and 5-HT_{3A} (−/−) mice (n = 9) mice. All mice initially received four consecutive days of saline treatment and exposure to the test chambers for 1-h locomotor trials before exposure to cocaine. Cocaine (10.0 mg/kg i.p.) was administered every other day (e.g. at 48-h intervals) for a total of six injections and locomotor trials were run immediately following dosing. All cocaine injections were administered immediately prior to the start of each locomotor session, in the familiar context of the testing room in close proximity to the locomotor chambers.

Locomotor responses to saline injections pre- and post-cocaine sensitization

Locomotor response to saline injection was tested 2 days following the final cocaine challenge trial in the wild-type (n = 7) and 5-HT_{3A} (−/−) mice (n = 9) that received repeated intermittent exposure to cocaine. The data were compared with the fourth saline control injection that preceded sensitization testing.

Drugs

Cocaine hydrochloride (Sigma-Aldrich, St Louis, MO, USA) was dissolved in 0.9% saline and administered intraperitoneally in a volume of 0.01 ml/g body weight. Drug solutions were prepared immediately prior to each administration.

Data presentation and analysis

Data are presented from locomotor trials that tested: (1) spontaneous locomotor behavior and habituation in a novel environment, (2) acute cocaine treatment, (3) repeated intermittent (every other day) injections of cocaine to induce sensitization and (4) response to saline injection pre- and post-development of cocaine sensitization to characterize conditioning effects of drug-

environment pairings. Horizontal distance traveled (cm) was recorded during 60-min trials for baseline locomotor testing and 15-min trials after drug administration. Data were analyzed by two-way repeated measures ANOVA with cocaine (dose or treatment day) and genotype as factors. Planned *post hoc* comparisons were conducted using the Tukey test, $P < 0.05$. All statistical analyses were conducted with SigmaStat (Systat Software, San Jose, CA, USA).

Results

Locomotor ability and effects of acute cocaine

Mice lacking the 5-HT_{3A}-receptor subunit showed normal spontaneous locomotor activity and habituation to the open field over 3 days of testing as compared with wild-type littermate controls (Fig. 1a). A trend toward reduced habituation by the 5-HT3A (−/−) mice was observed during day 1 of testing but did not result in statistically significant differences between genotypes. Acute treatment with cocaine (10–20 mg/kg i.p.) after habituation produced a dose-dependent increase in locomotor activity in all mice ($F_{2,68} = 38.423$, $P < 0.001$) (Fig. 1b). Wild-type and 5-HT_{3A} (−/−) mice displayed similar levels of locomotor response to acute cocaine treatment. No effects of genotype ($F_{1,68} = 0.0647$, $P > 0.05$) or genotype × dose interaction ($F_{2,68} = 0.321$, $P > 0.05$) were found, showing absence of a differential genotypic sensitivity to the locomotor-stimulating effects of acute cocaine treatment.

Locomotor effects of repeated intermittent cocaine

Figure 2 shows that repeated intermittent treatment with cocaine (10 mg/kg i.p.) resulted in significantly heightened locomotion over successive days in both wild-type and 5-HT_{3A} (−/−) mice ($F_{9,126} = 48.8$, $P < 0.001$). A significant genotype × day interaction was observed ($F_{9,126} = 2.9$, $P = 0.004$) indicating that genotypic differences in locomotor activity depended on treatment day. Follow-up multiple comparison procedures (Tukey Test) showed that 5-HT_{3A} (−/−) mice did not differ in response to saline or the first cocaine treatment but showed diminished cocaine-induced locomotor response to repeated cocaine exposure (Fig. 2; cocaine trial 2, 4, 5 and 6).

Effects of drug-associated conditioned cues

Figure 3 shows the locomotor response to saline injection during baseline conditions before cocaine exposure and 2 days following cocaine sensitization for both wild-type and 5-HT_{3A} (−/−) mice. Basal activity in response to saline injection did not significantly differ between the groups either when measured pre- or post-cocaine sensitization [group ($F_{1,31} = 2.709$, $P > 0.05$)]. The locomotor response to saline was significantly heightened when measured following the final period of cocaine sensitization [day ($F_{1,31} = 26.133$, $P < 0.001$)] as compared with the pre-cocaine baseline. This may suggest an anticipatory or conditioned effect to chronic cocaine exposure, produced by repeated pairings of cocaine with the specific environmental cues associated with the location in which the drug was administered. No group × day interaction was observed ($F_{1,31} = 0.513$, $P > 0.05$), indicating that both wild-type and 5-HT_{3A} (−/−) mice displayed similar degrees of hyperlocomotion in response to saline when tested post-cocaine sensitization.

Discussion

Behavioral sensitization to psychostimulant drugs is characterized by a progressive increase in locomotor behavior following repeated intermittent exposure. This drug effect has been studied extensively in animals as a model of behavioral and molecular adaptations that underlie aspects of drug addiction (Carlezon & Nestler 2002; Robinson & Berridge 1993, 2000; Thomas *et al.* 2001; Wolf 1998). Sensitization is thought to consist of components of induction and expression, which are believed to be temporally distinct and regulated by different

neurochemical and neuroanatomical systems (Pierce & Kalivas 1997; Vanderschuren & Kalivas 2000). Induction of sensitization refers to the adaptive changes that take place as a result of first drug exposures. These initial cellular events precede the enduring changes thought to underlie the expression of sensitization, which occurs after a period of drug withdrawal.

Results of the present study add to growing evidence that implicates serotonin receptor systems in the process of behavioral sensitization (Cunningham *et al.* 1992; Parsons & Justice 1993). In particular, 5-HT_3 receptor antagonists have been reported to attenuate locomotor stimulatory effects of both systemically administered cocaine (Costall *et al.* 1990; Reith 1990; Svingos & Hitzemann 1992) and direct infusion of dopamine into the NAcb (Costall *et al.* 1987). Other evidence indicates that coadministration of the 5-HT_3 antagonist on-dansetron with cocaine blocks the induction of cocaine sensitization (King *et al.* 1997). Similarly, 5-HT_3 immunoreactivity is downregulated in the shell of the NAcb during the induction of cocaine sensitization (Ricci *et al.* 2004), suggesting that initial adaptations to cocaine may involve activation of 5-HT_3 receptors. In the present study, targeted deletion of the 5-HT_{3A}-receptor subunit gene resulted in diminished locomotor response to repeated intermittent cocaine exposure in C57BL/6J mice. This effect appeared to be specifically related to blunted induction of cocaine sensitization in the 5-HT_{3A} (−/−) mice because these mutants displayed similar basal activity and locomotor response to acute cocaine treatment when compared with wild-type mice. These results suggest that full induction of cocaine sensitization requires the 5-HT_{3A}-receptor subunit.

It will be of interest in future studies to examine involvement of 5-HT_{3A} receptors in the expression of cocaine sensitization to determine if this system also regulates the progressive adaptations in brain and behavioral function after cocaine exposure. Evidence suggests that 5-HT_3-receptor antagonists can alter expression of cocaine sensitization but the effect may depend on when the antagonist compounds are administered in relation to cocaine treatment. For example, injections of the 5-HT_3 antagonist, ondansetron on the first 5 days of withdrawal from intermittent cocaine significantly blocked the expression of sensitization when tested after 7 days of withdrawal in rats (King *et al.* 1998). Alternatively, in another study, the 5-HT_3 antagonists MDL 72222, Y-25130 or ondansetron were administered before each repeated cocaine treatment (Szumlinski *et al.* 2003). Consistent with the results of our study in 5-HT_{3A} (−/−) mice, 5-HT_3 antagonists inhibited the progressive locomotor effects of cocaine during treatment. However, there was no effect of the antagonist treatment when expression of cocaine sensitization was tested 3 weeks later (Szumlinski *et al.* 2003). Together these studies suggest that 5-HT_3-mediated neuro-adaptations that occur during withdrawal are important for expression of behavioral sensitization to cocaine. Whether 5-HT_{3A} receptors mediate the withdrawal-associated changes remains to be determined.

In general, induction of behavioral sensitization to cocaine is thought to be mediated by cellular events in the ventral tegmental area (VTA), NAcb, and prefrontal cortex [reviewed by (Vanderschuren & Kalivas 2000)]. However, as the 5-HT_{3A}-receptor subunit has been found to predominate in the hippocampus, amygdala, cortex and NAcb (Tecott *et al.* 1993), loss of ligand-gated ion channel function in any or all of these limbic structures may have impacted development of cocaine sensitization in the 5-HT_{3A} (−/−) mice. Further research is needed to determine if 5-HT_{3A} receptors regulate the induction cocaine sensitization in a brain region-dependent manner.

One possible mechanism of action to explain the blunted development of cocaine sensitization following loss of the 5-HT_{3A}-receptor subtype is that 5-HT_3 receptors may modulate midbrain dopamine systems. *In vivo* microdialysis studies have confirmed that 5-HT_3 receptors regulate extracellular dopamine levels within the NAcb (Imperato & Angelucci 1989), and that agonists at the 5-HT_3 receptor increase (Chen *et al.* 1991), while antagonists inhibit accumbal dopamine

release (Carboni *et al.* 1989). Chronic cocaine treatment increases both dopamine and serotonin in the NAcb via interactions with and blockade of their transporters (Ritz *et al.* 1990), increasing the likelihood that these transmitters interact to modulate behavioral sensitization. Electrophysio-logical studies have shown that increased synaptic 5-HT inhibits firing of neurons in the NAcb (Henry *et al.* 1989). Also, treatment with LY-278584, a drug which displays high affinity and selectivity for rat and human brain 5-HT_3 receptors (Abi-Dargham *et al.* 1993; Wong *et al.* 1989), decreases the activity of dopaminergic neurons in the VTA (Minabe *et al.* 1991). Thus, interaction with dopaminergic systems is one potential mechanism of 5-HT_3 receptor modulation of the induction of cocaine sensitization.

Another potential mechanism by which deletion of the 5-HT_{3A} receptor may have blunted induction of cocaine sensitization is through modulation of glutamate transmission. A variety of studies implicate ionotropic *N*-methyl-D-aspartate (NMDA) receptors in the induction of cocaine sensitization (Druhan & Wilent 1999; Ida *et al.* 1995; Karler *et al.* 1989; Li *et al.* 1999; Pudiak & Bozarth 1993). Recent evidence indicates that repeated cocaine administration results in a down-regulation of NMDA NR1 and NR2B receptor subunits in striatum, NAcb and cortical brain regions (Yamaguchi *et al.* 2002), which is consistent with cocaine-induced increases in extracellular glutamate in brain regions that regulate sensitization (Kalivas & Duffy 1998; Smith *et al.* 1995; Williams & Steketee 2004). Other evidence indicates that 5-HT_3-receptor activation increases neural glutamate release (Ashworth-Preece *et al.* 1995; Funahashi *et al.* 2004). Thus, it is plausible that absence of 5-HT_3–mediated glutamate release may have resulted in blunted cocaine-induced sensitization in the 5-HT_{3A} (−/−) mice in this study. It would be of interest to determine if blockade of 5-HT_3-receptor function, or 5-HT_{3A} gene deletion, can prevent cocaine-induced increases in glutamate as seen in behavioral sensitization.

Another factor to consider when interpreting changes in behavioral sensitization is the role of conditioning that occurs when repeated drug effects are tested in a specific environment. Many studies have found evidence to suggest that environmental factors associated with drug exposure can influence behavioral sensitization (Browman *et al.* 1998; Partridge & Schenk 1999; Robinson *et al.* 1998). Specifically, groups of animals in which drug treatments are paired with the test environment express sensitization at a higher level than groups of animals that receive drug in an environment, which is novel and unassociated with the test conditions (Robinson *et al.* 1998). This issue is important to consider in the present study because 5-HT_3 over-expressing mice exhibit enhanced contextual conditioning (Harrell & Allan 2003), which suggests that 5-HT_{3A} (−/−) mice may have shown blunted sensitization because of impaired conditioning. This did not appear to be the case, however, because the 5-HT_{3A} (−/−) mice showed normal habituation to a novel environment and similar levels of drug-conditioned effects when tested with saline injection post-cocaine sensitization. Moreover, response to acute cocaine appeared to be equally attenuated in both genotypes after repeated saline injections (compare the acute response to cocaine in Fig. 1 to that seen in Fig. 2), which is consistent with evidence showing that locomotor response to cocaine is attenuated as a function of increasing exposure to the test environment after repeated saline injections (Todtenkopf & Carlezon 2006). These observations support the hypothesis that 5-HT_{3A} receptor–related neuroadaptations and not psychological factors, such as conditioning or stress responding to the drug or environment, formed the basis for the blunted development of cocaine sensitization in the 5-HT_{3A} (−/−) mouse. This is of particular relevance as our group has previously reported differences in anxiety-related behaviors in this mouse (Kelley *et al.* 2003).

Results of this study suggest that the 5-HT_{3A}-receptor subunit is required for the full induction of cocaine sensitization. This conclusion is based on the observation that mice carrying a null mutation for the 5-HT_{3A}-receptor gene were less responsive to the progressive increase in locomotor activity seen following repeated intermittent cocaine treatment. Although targeted

gene deletion has been used to create numerous null mutant mice that have been used to identify a variety of gene-behavior relations (Heisler *et al.* 1998; Hodge *et al.* 1999; Jones *et al.* 1998; Kelley *et al.* 2003; Saarelainen *et al.* 2003), use of the traditional gene knockout strategy raises several interpretive concerns including the potential role of developmental compensation and influence of mouse genetic background (Gerlai 2001). For example, null mice that lack the α_1 subunit of ionotropic $GABA_A$ receptors show developmental compensation in other molecular subunits of the receptor including decreased expression of $\beta_{2/3}$ and γ_2 subunits and increased expression of α_2 and α_3 subunits (Kralic *et al.* 2002). At present, there is no evidence available regarding potential compensatory changes in other 5-HT_3 molecular subunits in 5-HT_{3A} (−/−) mice. However, it appears that deletion of the 5-HT_{3A} subunit prevents the formation of functional 5-HT_3 receptors because the 5-HT_3 agonist m-Chlorophenylbiguanide (mCPBG) has no effect on the firing rate of peripheral nociceptors or cultured dorsal root ganglion neurons obtained from the 5-HT_{3A} (−/−) mice (Zeitz *et al.* 2002). Whether compensatory changes or genetic background contributed to the blunted sensitization seen in the 5-HT_{3A} (−/−) mice in this study remains to be determined; however, converging evidence from this study and reports cited above (i.e. King *et al.* 1997; Ricci *et al.* 2004) are consistent with the conclusion that 5-HT_3 receptors regulate neurobehavioral adaptations to repeated cocaine.

In summary, 5-HT_{3A} (−/−) mice showed blunted cocaine-induced locomotor sensitization in the absence of differential motor ability, acute response to cocaine or response to the environment paired with cocaine. These data suggest that the 5-HT_{3A}-receptor subunit modulates adaptive changes in behavior that occur following repeated cocaine exposure. The precise mechanism(s) underlying blunted development of cocaine sensitization found in the 5-HT_{3A} (−/−) mice requires further study. However, it is apparent that alterations in neural functioning and not conditioning effects related to repeated cocaine exposure underlie these differences. The current data support a role for the 5-HT_{3A}-receptor subunit in the induction of cocaine sensitization and suggest that agents with selectivity for the 5-HT_{3A}-receptor subunit may hold therapeutic potential in the treatment of psychostimulant abuse.

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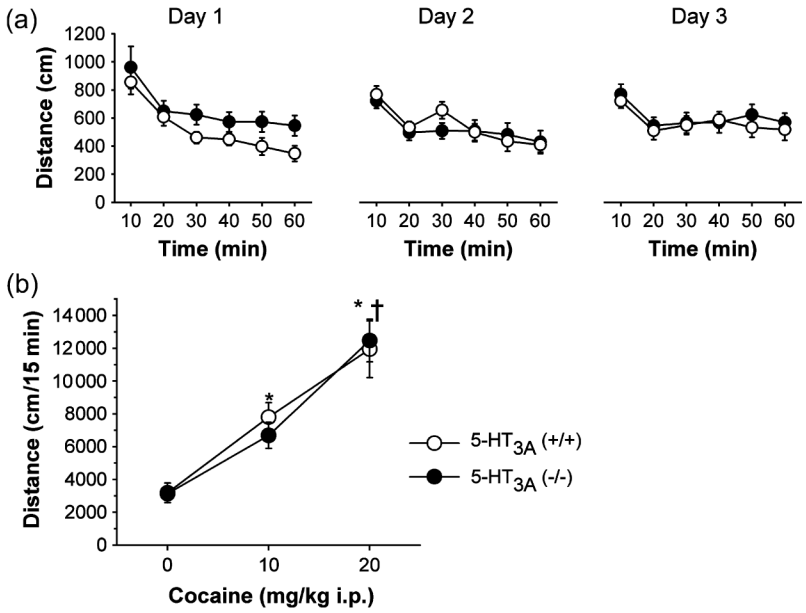

Figure 1. Deletion of the 5-HT$_{3A}$ receptor subunit does not affect motor ability or acute locomotor response to cocaine

(a) Spontaneous locomotor activity and habituation to a novel open field environment measured over three consecutive days. (b) Locomotor response to acute cocaine (0–20.0 mg/kg i.p.) measured during 15-min sessions. Data are plotted as mean horizontal distance traveled (cm) (±SEM), for wild-type (filled circles, $n = 10$), and 5-HT$_{3A}$ (−/−) mice (open circles, $n = 13$). *Significantly different from saline control; †significantly different from cocaine (10.0 mg/kg), Tukey test ($P < 0.05$).

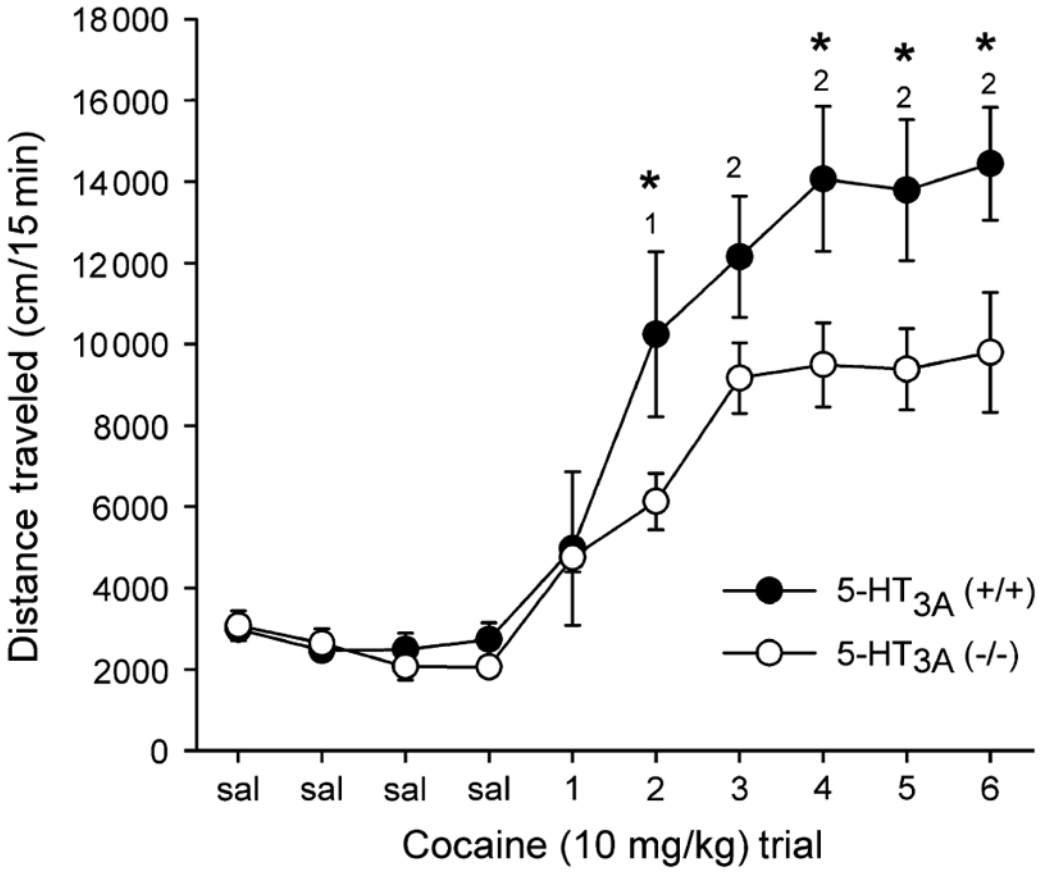

Figure 2. Targeted deletion of the 5-HT$_{3A}$ receptor subunit blunted the development of cocaine locomotor sensitization in response to chronic intermittent administration of cocaine (10 mg/kg i.p.)

Data are presented as mean distance traveled (cm) (±SEM), for 15-min locomotor trials in wild-type (filled circles, $n = 7$), and 5-HT$_{3A}$ (−/−) mice (open circles, $n = 9$) across four initial saline trials (S1–S4) and six intermittent cocaine exposures.

*Significant difference among genotypes at corresponding x-axis point. 1, wild type significantly different from acute cocaine (trial 1); 2, both genotypes significantly different from acute cocaine, Tukey test ($P < 0.05$).

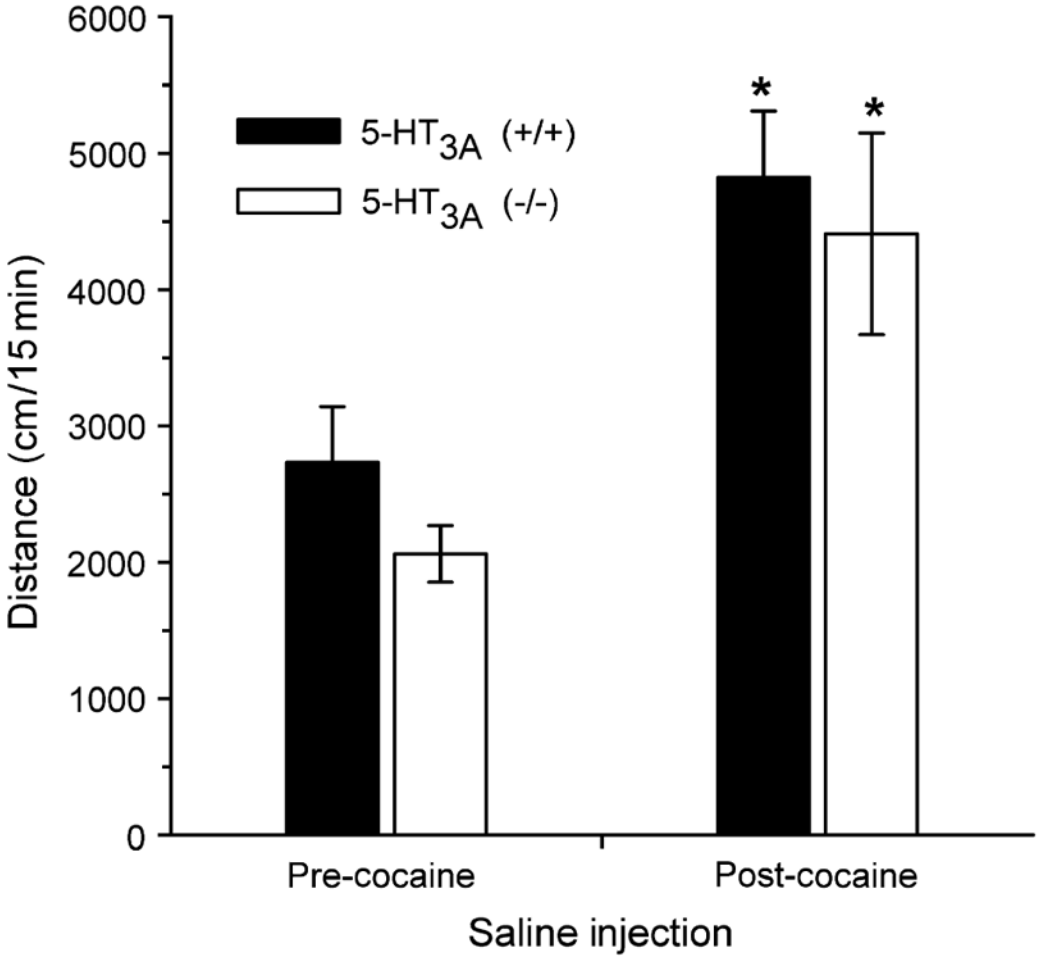

Figure 3. No genotypic difference when mice were exposed to the drug-paired environment

The locomotor response to saline injection (0.01 ml/g) at baseline, prior to cocaine exposure (left panel) and 2 days post-cocaine sensitization (right panel) in wild-type (filled bars, $n = 7$) and 5-HT_{3A} (−/−) mice (open bars, $n = 9$). Data are presented as mean distance traveled (cm) (±SEM), for 15-min locomotor trials. Two-way ANOVA showed an effect of treatment ($P < 0.05$) but not genotype, with both genotypes showing significantly heightened activity post-cocaine.* Significantly different from pre-cocaine within genotype, Tukey test ($P < 0.05$).